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AGGREGATION OF LIPOSOMES BY DEXTRANS OF HIGH MOLECULAR WEIGHT

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SUMMARY: Sonicated dispersions (liposomes) of natural and synthetic phospholipids are aggregated reversibly by Dextrans 40, 110 and 500. The dextran concentration required for aggregation is dependent on chain length, lipid composition of the liposome and, for ionically-charged phospholipids, the ionic strength of the medium. The results indicate that adsorption of dextrans to the erythrocyte surface can occur by interaction with surface phospholipid substituents.

Aggregation of erythrocytes by high molecular weight dextrans has been studied systematically (1,2) and is believed to result from adsorption of the neutral polymers to the cell surface. The surface membrane groups involved in the adsorption, however, are unknown (1), prompting us to explore the possibility that the phospholipid substituents of the outer membrane surface are responsible. The experiments below demonstrate that sonicated dispersions (liposomes) of natural and synthetic phospholipids do interact with dextrans and aggregate as a result. This interaction identifies one mechanism for dextran adsorption at the erythrocyte surface.

METHODS

Liposome preparation. Sonicated dispersions of natural or synthetic phospholipids were prepared as previously described (3), generally from suspensions of 15 mg/ml of lipid in 150 mM

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KCl-10 mM Tris buffer (pH 7.4). Human erythrocyte membrane lipid was extracted from ghost membranes (3), using the procedure of Folch et al. (4).

Aggregation studies. Liposome suspensions, freshly prepared, were diluted 1/10 with the KCl-Tris solution and the optical density at 500 nm monitored in a Beckman DU spectrophotometer. To the experimental sample small aliquots of dextran solution (100 mg/ml in the KCl-Tris) were added with mixing and thereafter sufficient time (approximately 2 min) allowed to stabilize each reading.

Materials. Egg lecithin (Type IX), brain phosphatidyl serine and synthetic dioleoyl phosphatidyl choline were purchased from Sigma Chemical Co. (St. Louis, Mo.). Dextrans 40, 110 and 500, with weight average molecular weights of 4.4×10^4, 1.1×10^5 and 4.5×10^5, respectively, were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

RESULTS

Addition of Dextrans 40, 110 or 500 to suspensions of egg lecithin liposomes results in turbidity which can be monitored by optical density measurements, as shown in Fig. 1. Microscopic examination at 5000 X magnification shows that the turbidity is due to aggregation of individual liposomes. The process is reversible; dilution of the dextran disaggregates the liposomes and clarifies the suspension. The increase in optical density with dextran concentration (Fig. 1) follows a sigmoidal curve and the apparent half-maximal concentrations for Dextrans 40, 110 and 500 respectively, are approximately 1.6×10^{-3} M, 5.5×10^{-4} M and 9.0×10^{-5} M. This inverse relationship between the concentration required for aggregation and the dextran chain length has also been observed in erythrocyte aggregation (5).

The concentration of Dextran 500 required for aggregation depends on the composition of the liposome. When sonicated dispersions of cholesterol/egg lecithin (molar ratio 0.95, similar to that in human erythrocyte membrane lipid) were tested, the resulting curve of increasing optical density was

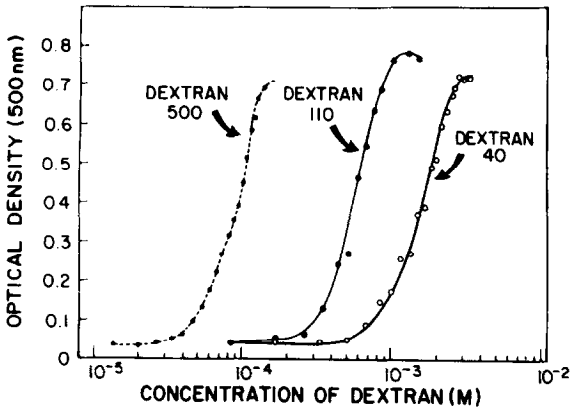

Fig. 1. Effects of dextran concentration on optical density of suspensions of egg lecithin liposomes.

shifted toward lower dextran concentrations, with an apparent half-maximal concentration of approximately 3.5×10^{-5} M. Liposomes of human erythrocyte membrane lipid are also aggregated by Dextran 500. The curve of increasing optical density is biphasic, with a rapid rise in the dextran concentration range 1.8×10^{-5} M to 2.3×10^{-5} M and a slower rise thereafter. It is noteworthy that the foregoing concentrations of Dextran 500 required for liposome aggregation are comparable to the concentration needed for optimal erythrocyte aggregation, approximately 7×10^{-5} M (5).

Surface charge influences erythrocyte aggregation (1). We examined the effects of surface charge in liposomes by comparing sonicated dispersions of synthetic dioleoyl lecithin (98% pure) with those of a dioleoyl lecithin/phosphatidyl serine mixture (molar ratio 4/1). Each type of liposome was prepared and tested with Dextran 500, both in the usual KCl-Tris solution (ionic strength 160 mM) and in 1/10 the concentration (ionic strength 16 mM). The results in Fig. 2 indicate that the pure lecithin liposomes, presumably electrically neutral, are more

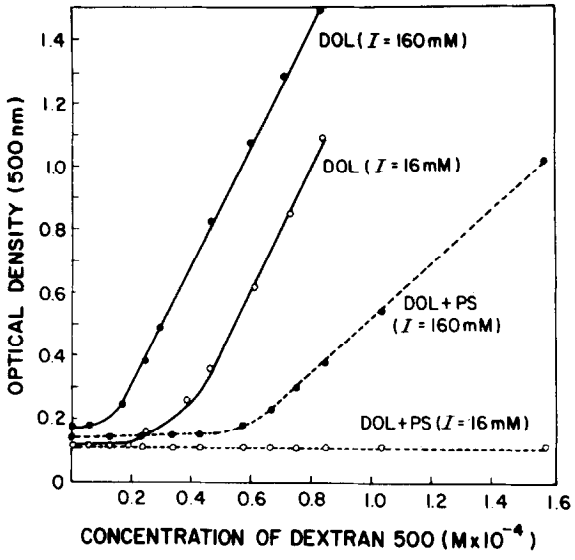

Fig. 2. Effects of Dextran 500 on optical density of liposome suspensions of dioleoyl lecithin alone (DOL) or dioleoyl lecithin plus phosphatidyl serine (DOL + PS). Each kind of liposome was prepared and tested in KCl-Tris of ionic strength 160 mM and 16 mM.

readily aggregated by dextran than are those containing the negatively-charged phosphatidyl serine molecules. Decreasing the ionic strength increased somewhat the dextran concentration required to aggregate the pure lecithin liposomes. (Some of this effect probably derives from ionically-charged impurities in the lecithin preparation, inasmuch as the effect of ionic strength was exaggerated with less pure egg lecithin liposomes.) Aggregation of the liposomes containing phosphatidyl serine, however, was completely abolished in the low ionic strength medium.

DISCUSSION

The concentrations of dextran required for liposome aggregation, the variations with dextran chain length and the interrelationship of surface charge and ionic strength of the medium

are all similar to phenomena observed in studies of erythrocyte aggregation (1). It is reasonable to conclude, therefore, that the outward-facing phospholipid substituents of the erythrocyte membrane provide sites for the adsorption of dextran molecules. This conclusion does not exclude other surface sites—and we have found that dextrans reversibly inhibit D-glucose transport across erythrocyte membranes, presumably by binding to exofacial transport sites. Binding to the transport sites is not required for erythrocyte aggregation, however, because prior alkylation of the transport sites by glutathione maleimide, an impermeant inhibitor of the transport (6), fails to prevent dextran-induced aggregation of erythrocytes.

In addition to defining one mechanism for the adsorption of dextrans to the erythrocyte surface, the methods employed here should be useful in further studies of the chemical and physical properties of these model membrane preparations.

ACKNOWLEDGEMENTS

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